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Results: In comparison to control MCF7 cells, jadomycins G, DNV, B and N were equally toxic to ABCB1-overexpressing MCF7 cells; jadomycins DNV, SPhG and N were equally toxic ABCC1-overexpressing MCF7 cells; and only jadomycin N was equally toxic to ABCG2-overexpressing MCF7 cells. None of the jadomycin analogues inhibited the efflux of ABCB1, ABCC1 or ABCG2 probe substrates in transport assays.
Conclusion: The ability of jadomycins to retain cytotoxic activity in the corresponding drug resistant MCF7 cells stems from their ability to circumvent interactions with the ABCB1, ABCC1 and ABCG2 drug efflux transporters.

951 Molecular Studies of Mechanisms of Drug Resistance in Malignant Cells With Focus on Lymphomas

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Background: Multidrug resistance to anticancer drugs is a major cause of chemotherapy failure in cancer patients. In efforts to find novel approaches to inhibit proliferation and induce apoptosis in lymphoma cells, we examined in both Hodgkin and non-Hodgkin lymphoma cell lines, the action of naturally occurring compound curcumin which is nontoxic and has a variety of therapeutic properties including anti-oxidant, analgesic, anti-inflammatory and antiseptic activity.
Methods: Both Hodgkin and non-Hodgkin cells were pre-treated with curcumin followed by exposure to doxorubicin or vincristine and the effect on cell growth was determined. Cytotoxic effects and determination of apoptotic attributes upon curcumin treatment were analyzed using flow cytometry assays.
Results: The current study demonstrates that curcumin has the ability to decrease cell viability and it is due to its capacity to decrease cell proliferation by causing cell cycle arrest in G2/M phase, and by inducing apoptotic cell death. curcumin pre-treatment followed by exposure to doxorubicin or vincristine increased apoptosis as indicated by annexin V staining. It is shown that curcumin is much more effective on lymphoma cell lines in compare to doxorubicin or vincristine.
Conclusion: We have demonstrated that curcumin is an efficient inducer of apoptosis in lymphoma cell lines, meriting its further evaluation in vivo. The observed effects combined with the well established pharmacological safety of curcumin, provides rationale for the potential use of curcumin as a new therapeutic agent for patients with Hodgkin and non-Hodgkin lymphomas.

952 Uptake and Immunomodulatory Effect of Pegylated Liposomal Doxorubicin Nanoparticles on Human Macrophages

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Introduction: The pegylated liposomal doxorubicin (PLD) has been widely accepted in treatment of various cancers. However, the composition of two currently marketed PLD nanoparticles differs in structure and composition of lipids, and their differential effects remain unknown. Macrophages of the mononuclear phagocyte system are pivotal in determination of PLD clearance in vivo. The aim of this study was to compare the effect of these two PLDs on drug uptake, cell viability, morphology and immune function of human macrophages.
Material and Method: Two PLD nanoparticles were used in this study. The major difference between Lipo-dox® (PLD-D) and Caelyx® (PLD-H) is that their phospholipid bilayers are composed of distearoyl phosphatidylcholine (DSPC) and hydrogenated soybean phosphatidylcholine (HSPC), respectively. Human $CD14^+$ monocytes were isolated from peripheral blood to prepare macrophages for this study. Comparative assays included: flow cytometry for detection of doxorubicin penetration into cells, MTT for cell viability, Trypan blue exclusion for cell membrane integrity, Liu's stain for morphologic evaluation, inactivated yeast co-culture for phagocytosis.
Results and Discussion: The uptake of PLD-H was rapidly detected at 10 min and kept increasing to 4 h followed by a decline thereafter, whereas that of PLD-D had similar profile with much less doxorubicin fluorescence detected, indicating a greater amount of doxorubicin retention of PLD-H. PLD-H, at higher concentration, decreased the viability and impaired cell membrane integrity of macrophages with an extent greater than PLD-D. The morphological observation showed a more extensive necrosis in PLD-H-treated macrophages. The phagocytosis function of macrophage was inhibited with a greater extent in PLD-H-treated macrophages.
Conclusion: To human macrophages, PLD containing HSPC may cause greater amount and longer retention of doxorubicin in cells, greater toxicity and more profound dysfunction than that containing DSPC. Whether this differential effect correlates to the clinical outcome needs to be extensively investigated by performing in vivo experiments or clinical trials.

953 Discovery of a New Inhibitor of P53/MDM2 Interaction Using a Yeast Target-based Screening Strategy

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The p53 tumour suppressor is a major regulator of cell proliferation and death. In tumours that retain a wild-type (wt) p53, the activity of this protein can be inhibited by the endogenous negative regulator MDM2. In this case, inhibitors of p53/MDM2 interaction have been considered promising drugs for cancer therapy [1,2].
In this work, yeast assays consisting of *Saccharomyces cerevisiae* cells co-expressing human wt p53 and MDM2 were used for the screening of inhibitors of p53/MDM2 interaction. In our experimental approaches in yeast, inhibitors of p53/MDM2 interaction, such as Nutlin-3A, revert the inhibitory effect of MDM2 on p53-induced growth inhibition/cell cycle arrest as well as on p53-dependent transcriptional activity of a reporter gene (described in [3]). A chemical library of small-molecules inhibitors of tumour cell growth was tested and the small-molecule LEM1 was identified as inhibitor of p53/MDM2 interaction. The validation of the molecular mechanism of action of LEM1 was carried out in two human tumour cell lines derived from breast cancer (MCF7) and colon carcinoma (HCT116 $p53^{+/+}$) by performing gene reporter assays and by analysis of p53, p21 and Bax protein levels as well as caspase activation by Western blot. The results obtained confirmed that 10 μM LEM1 treatment stimulated p53-dependent transcriptional activity, led to p53 protein stabilization, increased p21 and Bax protein levels, and induced caspase-7 activation in human tumour cell lines. Notably, these effects were not observed in the HCT116 $p53^{-/-}$ derivative cell line.
In conclusion, an efficient yeast-based high-throughput screening strategy was established to search for inhibitors of p53/MDM2 interaction. Most importantly, with this approach, LEM1 was discovered as an inhibitor of p53/MDM2 interaction. LEM1 represents a promising small-molecule to be further explored as anticancer drug and/or as a lead compound toward the synthesis of more potent and selective inhibitors of p53/MDM2 interaction.

Reference(s)

[1] Wang W and Hu Y. Med. Res. Rev. 2011; DOI: 10.1002/med.20236.
[2] Yu Q. *Drug Resist. Updat*. 2006; 9(1): 19–25.
[3] Andreotti V et al. Plos One. 2011; 6(6): e20643.
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954 Immunophenotype and CD99 Expression Patterns of Human Mesenchymal Stem Cells and Their Implication in Ewing Sarcoma Genesis, Biology and Treatment

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Introduction: Ewing Sarcoma (ES) is a malignant tumor affecting mainly children and young adults, arising especially in bone and soft tissue and characterized by the presence of a chromosomal translocation responsible for the transcriptional deregulation of target genes such as the membrane receptor CD99. The origin of ES has long been the focus of intensive research, however, recently a Mesenchymal Stem Cell (hMSC) origin has been assumed as the most probable.
Despite prior evidence suggesting a hMSC origin of ES, hMSC from ES patients have not been sufficiently investigated. Herein, we compared a large